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Serial measurements of GFR in infants using the continuous iothalamate infusion technique

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Serial measurements of GFR in infants using the continuous iothalamate infusion technique. We undertook a preliminary study to determine if a clinical trial was feasible that would compare the effect of a low protein vs a control formula on GFR and growth in infants with congenital renal insufficiency (C_{Io} < 55 ml/min/1.73 m^2). In this report from the Infant Diet Protein Study, we describe validation of a method using the plasma clearance of iothalamate (C_{Io}) as an estimate of glomerular filtration rate (GFR) and results of the preliminary study relating to renal function. The plasma C_{Io} method was validated as an accurate estimate of GFR by showing it to be the same as the plasma clearance of inulin (C_{In}). In the preliminary study infants who qualified for the study were randomly assigned to a low protein or control formula and were followed from 8 to 18 months of age. C_{Io} was measured at 8, 14 and 18 months of age in 21 of the infants and at 8 and 18 months of age in all twenty four infants that entered the study. Average absolute GFR in the 24 infants increased in the 10 month period from 5.3 ± 2.7 to 7.6 ± 4.5 ml/min. The percent increase in GFR was no different in infants whose GFR at 8 months of age was severely reduced from those whose GFR was only moderately reduced. When adjusted for age and body size, GFR did not change. Change in mean C_{Io} or serum creatinine (S_{Cr}) from 8 to 18 months of age between the infants in each diet groups was not different. We conclude that a clinical trial enrolling more infants and extending the study period is necessary to evaluate dietary protein effect.

There is a consensus that most patients with renal disease and a glomerular filtration rate (GFR) that is less than half normal will sustain progressive loss of renal function regardless of the cause [1]. This appears to be true for children with dysplasia, obstructive uropathy, cortical necrosis, or polycystic kidney disease, in whom there is no active disease causing further injury [2, 3]. These studies show that the rate of progression, as measured by loss of GFR per year, varies widely from patient to patient, but that in most patients progressive loss proceeds to end-stage renal disease.

A similar process is well documented in rats with reduced renal function secondary to renal ablation or disease. A low protein diet slows progression of this process in rats [4, 5]. There is evidence from clinical studies suggesting that this may be true in humans, both adults [6] and children [7, 8], who have chronic renal insufficiency (CRI). However, no controlled study in children has been published that tested this hypothesis; the evidence from a meta-analysis of controlled studies in adults indicates a benefit [9]. We therefore designed a multicenter controlled clinical trial to evaluate this question in infants with CRI. A protocol was developed and a feasibility study was undertaken.

The first problem to be addressed in testing the effect of a low protein diet on renal function is how to accurately assess progression as measured by a change in GFR. Neither serum creatinine (S_{Cr}), its reciprocal [10, 11] nor creatinine clearance (C_{Cr}) [12] is sufficiently sensitive for measuring rate of GFR decline in trials lasting only one to three years. A second problem, unique to children, is that GFR in normal children is increasing. The time of greatest increase is during the first two years of life [13].

We adapted a method for estimating GFR using plasma steady-state clearance methodology [14], substituted iothalamate for inulin as the marker and validated it. We then studied serial changes in growth and in the C_{Io} in infants with CRI (C_{Io} < 55 ml/min/1.73 m^2) from 8 to 18 months of age when they were fed formulas having different levels of protein. We evaluated the change in growth and in GFR during this period of rapid growth and examined how severity of reduction in GFR at eight months of age influenced this change. In this report we

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describe validation of the C_{Io} method and changes in serial GFR in infants who completed the 10 month period of study while receiving different protein intakes.

Methods

Validation of iothalamate clearance

We compared the plasma C_{Io} with that of inulin because the C_{In} is the reference measure of GFR. Eleven patients and one volunteer were given loading doses of inulin (40 mg/kg) and iothalamate meglumine (Conray 60 made by Malinkrodt Medical Co, St. Louis, Missouri, USA) (2 mg/kg). The loading dose and sustaining infusion were calculated to attain a serum level of 0.2 mg/ml for inulin and 0.01 mg/ml for iothalamate. We estimated the patient's GFR (est GFR) for each clearance study using the height/S_{Cr} formula [15] (ml/min/1.73 m^2) and adjusted it to the subject's own surface area (SA).

$$\text{Est GFR} = \frac{k \times Ht}{S_{Cr}} \times \frac{\text{Pts SA}}{1.73\ m^2} \quad (1)$$

In this study we used 0.45 as the constant k throughout. SA was derived from the standard nomogram or calculated from the height, weight formula of Haycock and colleagues [16]: $SA = 0.024265 * Wt^{0.5378} * Ht^{0.3964}$.

A minimum infusion time to reach steady-state serum iothalamate (S_{Io}) was calculated on assumptions that a loading dose would elevate S_{Io} to between 0.6 and 2.4 of steady state and that the C_{Io} would be greater than 60% of est GFR. Under these conditions 2.5 estimated half lives (t½) would be sufficient to reach steady state (100 ± 10%) so long as the estimated t½, calculated on 0.6 est GFR, is longer than the true t½ of the C_{Io}.

$$\text{Min infus time (hr)} = 2.5 * \frac{\ln 2}{\dfrac{0.6 \times \text{est GFR (ml/min)}}{\text{ECFV (ml)}}} \times \frac{1}{60} \quad (2)$$

Blood samples were drawn prior to the loading dose, after the minimum infusion time, and one hour later.

The C_{Io} was calculated from the following equation:

$$C_{Io}\ \text{(ml/min)} = \frac{\text{infusate Io (mg/ml)}}{\text{S.Io (mg/ml)}} \times \text{pump speed (ml/min)} \quad (3)$$

Feasibility study

The protocol was approved by the Committee on Human Research for each participating institution. Each guardian signed a consent form after reading an illustrated brochure prepared for the study and discussing the protocol with their physician and research nurse.

Protocol. The feasibility study involved twelve centers. Patients who met the following criteria entered an adjustment phase: they were less than six months study age (birth date adjusted for those who were premature), weighed more than 1500 g at birth, and had a $S_{Cr} > 0.5$ mg/dl resulting from renal dysplasia, obstructive uropathy, polycystic disease or perinatal cortical necrosis. The adjustment period ran to eight months study age during which each patient was managed in accordance with a treatment protocol directed to correcting electrolyte, acid base and/or mineral imbalances and to assure, insofar as possible, an adequate energy intake. Patients, during this period, were fed a formula with an intermediate (8%) protein: energy (P:E) ratio.[1] At 8 months study age, if S_{Cr} remained > 0.5 mg/dl, a C_{Io} study was done. If the C_{Io} was less than 55 ml/min/1.73 m^2 they then entered the study phase and were randomly assigned to receive either a low protein (P:E 5.6%)[1] or control (P:E 10.4%)[1] formula. They were continued on the assigned formula until they reached 18 months of age when the study ended. During this period standardized anthropometric, clinical, and laboratory data were collected every two months and C_{Io} studies were repeated at 14 and 18 months.

Average calorie and protein intake were estimated each month from diet records recorded three days each week every other week. Calorie intake was recorded as percent normal relative to Recommended Dietary Allowance (RDA) for height, and protein intake as g/kg/day. Protein intake also was monitored using the ratio of serum urea nitrogen to serum creatinine (S_{UN}:S_{Cr}).

The clearance protocol used in the feasibility study was as follows: The loading dose and infusion rate of iothalamate were calculated as described in the validation study. The est GFR used in setting up the clearance study was derived from the S_{Cr} obtained in the individual investigator's institution. Minimum infusion times, derived from the equation described above, were set as follows:

4 hr when the est GFR was ≥ 2 ml/min/kg body weight
8 hr when the est GFR was between 1 to 2 ml/min/kg
16 hr when the est GFR was between 0.5 to 1.0 ml/min/kg
24 hr when the est GFR was < 0.5 ml/min/kg

A blank (S_0), a one hour post-loading dose (S_1) and, after the minimum infusion time, two final blood samples (S_2, S_3) were obtained. S_2 usually was obtained shortly after the minimal infusion time, and S_3 30 to 60 minutes later. Sometimes S_3 was obtained on the same draw as S_2 but was treated separately. The average of S_2 and S_3 was used as the steady state S_{Io}. S_1 was used to check on the approximate accuracy of the loading dose and to compare with S_{Io}; if the ratio of S_1 to S_{Io} fell outside the limits of 0.6 and 2.4, we declared the study unsatisfactory. This happened twice owing to dilution errors in making up either the priming or sustaining infusions.

An untimed urine sample was obtained following the draw for S_2 in 48 studies; iothalamate, creatinine and albumin were determined on both the steady state serum (S_2 and S_3) and this urine. From these the true C_{Cr} and the fractional clearance of albumin (Θ Alb) were determined.

Analyses of serum, urine and infusate samples for iothalamate, creatinine and albumin were performed in the central (UCSF) laboratory. Samples chilled with frozen gel (4°C) were shipped express delivery in styrofoam containers to the central laboratory.

[1] Formulas were prepared through the courtesy of Ross Laboratories, Columbus, Ohio, USA, and met standards for infant formulas as set forth in FAO/WHO/UNU technical bulletin 724.= and the Committee on Nutrition of the American Academy of Pediatrics.

There was good agreement between the S_{Cr} results from the individual centers and those of the central lab. In only eight instances was the difference greater than 0.2 mg/dl and in only two was it greater than 0.3 mg/dl. When either occurred, the analyses were repeated in the central lab, and the average of all the results from the central lab were entered as central lab S_{Cr}. The central lab S_{Cr} values were used in comparing the calculated C_{Cr} values with those of C_{Io} and in comparing S_{Cr} and est C_{Cr} results from patients in the different diet groups.

Laboratory methods. Plasma ultrafiltrate for iothalamate analysis was prepared with Amicon centrifuge filters. Iothalamate was determined by HPLC using a reverse phase C18, 5 μ ultrasphere Beckman column and a water; acetonitrile; phosphoric acid (990:10:0.5) buffer at pH 2.65; 20 μl of plasma ultrafiltrate were injected onto the column. The peak was read at 229 Å using a Waters UV detector [17]. Retention time was nine minutes. The B_0 or blank values were uniformly low. Differences between duplicates of 20 samples analyzed blinded on different runs was less than 2%.

Inulin was measured by the anthrone method [18]. Serum and urine creatinine were measured using Sigma kit 555A. Serum albumin was measured by the Brom Cresol Green binding method read at 600 nM. Urine albumin was determined by nephelometry; it was sensitive to detect albumin concentrations of 6 mg/liter or greater.

Measured C_{Cr}. The C_{Cr} and C_{Io} were compared in 45 clearance studies where $(U/P)_{Cr}$ and $(U/P)_{Io}$ were both measured. C_{Cr} was calculated by the following equation:

$$C_{Cr} = \frac{(U/P)_{Cr}}{(U/P)_{Io}} \times C_{Io} \quad (4)$$

Albumin excretion. The excretion rates of albumin were expressed as Θ Alb and as U_{Alb}/U_{Cr}. The Θ albumin was calculated by the following equation:

$$\Theta\ \text{Alb} = \frac{[U/P]_{Alb}}{[U/P]_{Io}} \quad (5)$$

Statistical analysis

To test for trend in GFR measures at study ages 8, 14, and 18 months, univariate repeated measures analysis of variance was applied to absolute and log-transformed values [19]; orthogonal linear and quadratic contrasts were tested. For analyses of these measures with respect to study age, log-transformed values were used in order to stabilize variability. Tests for appropriateness of the univariate repeated measures analysis, that is, of sphericity of the contrasts, and of univariate normality of log GFR were applied. One-sample *t* or signed rank tests were applied to untransformed absolute and relative change scores. Differences in location between groups were tested using two-sample *t* or median tests. In some instances, two-group analyses of covariance were used with parallel or separate slopes on the covariate; equality of slopes and regressions between groups was tested. Kendall rank correlations were also tested. The SAS system [20] was used, with linear model analyses performed by the GLM procedure.

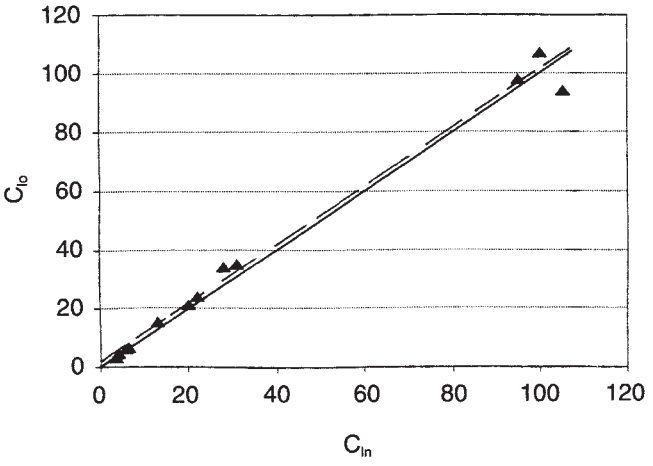

Fig. 1. *Comparing C_{Io} vs. C_{In} in 12 subjects, 11 patients and/or volunteers from the University of California, San Francisco (UCSF), and 1 patient from the University of Texas, Houston.* The regression of C_{Io} on C_{In} is as follows: $C_{Io} = 1.07 + 0.98 * C_{In}$. The mean and SD of the mean difference is 0.5 ± 1.0; $r = 0.99$.

Results

Validation of clearance method

The relation of the C_{Io} to the C_{In} observed in the validation study is illustrated in Figure 1. The differences between the C_{Io} and the C_{In} were not clinically or statistically significant ($P \geq 0.1$ by paired *t*-test and linear regression on both absolute and log transformed data).

Feasibility study

There were three technical failures in the 72 clearances done on the 24 patients who completed the study. The C_{Io}/est GFR using S_{Cr} data from the respective individual clinical lab was computed in 67 of the 69 successful studies. In only three instances was C_{Io} less than 60% of the est GFR. The average and SD of the ratio of GFR/est GFR was 0.97 ± 0.31; the coefficient of variation was 28%. Results using central lab S_{Cr} data were very similar.

Changes with growth. The averages and distributions of the absolute GFR (C_{Io}), GFR % nl for age and weight, and GFR adjusted to SA (ml/min/1.73 m^2) for studies of the 21 children who had successful clearances measured at 8, 14 and 18 mo are illustrated in Figure 2 A–C, respectively. Differences between study ages in means of log-transformed absolute GFR were highly significant ($P = 0.0002$), with a significant positive linear trend ($P = 0.004$). Thus mean absolute GFR increased significantly as the children grew. (For these repeated measures analyses, tests of sphericity were not significant ($P > 0.9$), and in general the assumptions appeared to be quite well satisfied.) For GFR values, whether expressed as log transformed % nl or adjusted to 1.73 m^2, the differences in means at the different study ages were not significantly different (% nl, $P = 0.17$; ml/min/1.73 m^2, $P = 0.12$).

The individual changes in GFR from 8 to 18 months were examined in more detail using paired clearances that were available in all 24 children (Table 1). Absolute GFR increased, both in terms of absolute change (mean increase = 2.3 ml/min; $P = 0.0009$) and relative change (mean increase = 48%; $P =$

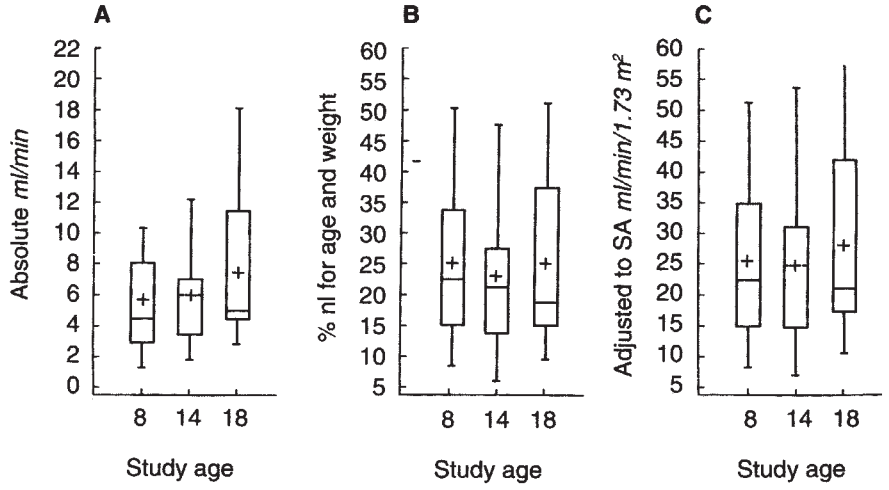

Fig. 2. *The average and distribution of C_{Io} for 21 patients studied at 8, 14 and 18 months of age.* **(A)** Absolute values which increased with age, **(B)** % nl values for age and weight which remained unchanged, and **(C)** values adjusted to 1.73 m^2 body surface area which also remained unchanged. The + is the mean, the middle line is the median, and the other horizontal lines are the 25 and 75 percentile lines. The vertical lines describe the range.

Table 1. Summary statistics (mean ± SD) of the differences in clearances between patients on the control and low protein diet at 8 and 18 months

	Age	Control $N = 13$	Low $N = 11$	P
GFR	8	5.7 ± 2.6	5.0 ± 2.5	0.50
(C_{Io})	18	*8.5 ± 4.6*	*6.6 ± 4.5*	0.28
ml/min	change	2.8 ± 2.2	1.6 ± 3.6	0.34
	8	1.2 ± 0.6	1.3 ± 0.4	0.66
S.Cr	18	*1.2 ± 0.7*	*1.6 ± 0.8*	0.22
mg/dl	change	0 ± 0.3	0.3 ± 0.6	0.15
est GFR	8	6.1 ± 2.6	5.0 ± 2.2	0.29
(Equation 1)	18	*9.8 ± 5.2*	*6.0 ± 2.3*	0.039
ml/min	change	3.1 ± 3.1	1.0 ± 1.1	0.012

0.0002). Absolute GFR increased by 20% or more in 15 of the 24 children; it decreased by more than 20% in only one. The relative increase was as great in those infants whose GFR was less than 25% nl at eight months of age as it was in those whose GFR at that age was 25 to 50% nl. The average GFR expressed either as % nl for age and weight or adjusted to 1.73 m^2 body surface area did not change significantly from 8 to 18 months of age. Changes in % nl GFR from 8 to 18 months were not significantly rank correlated with values at eight months ($P > 0.4$). While change in est GFR (formula of Equation 1) was statistically significant, errors in transforming the ht/S_{Cr} to est GFR data preclude giving clinical significance to this finding.

Comparing results between study groups

Details of the nutrition and growth data are described in a separate report [21]. The calorie intake of the two groups each averaged 92% RDA for height. The average protein intake for the low and control protein groups, respectively, were, over the period of study 1.6 ± 0.3 and 2.4 ± 0.4 g/kg/day; the average S_{UN}:S_{Cr} ratios were similarly different (low:control diet protein = 0.67; low:control S_{UN}/S_{Cr} = 0.61). Differences between the diet groups for GFR (C_{Io}) and S_{Cr} at 8 and 18 months and changes (18 to 8 months) were examined in the twenty-four patients using central lab values. The differences were not significant. The overall change in C_{Io} between the groups from 8 to 18 months also was not significant (Table 1).

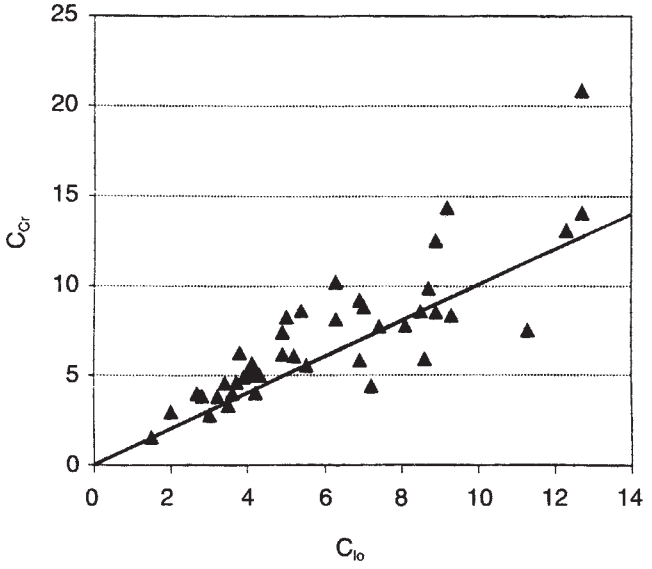

Fig. 3. *Comparing calculated C_{Cr} with C_{Io}.* The solid line is the line of identity. C_{Cr} is greater than GFR (C_{Io}). The difference is due to tubular secretion of creatinine (ts Cr) which varied considerably but averaged 15% of filtered creatinine.

C_{Io} versus measured C_{Cr}. When we compared the measured C_{Cr} calculated from U/P ratios of creatinine and iothalamate with C_{Io} (Equation 4), the slope was greater than 1.0 (Fig. 3). The fraction of creatinine that was secreted, (ts Cr), averaged 15 ± 27% (mean ± SD; ts Cr > 0; $P < 0.01$).

Albumin excretion. The Θ albumin and the urinary albumin related to urinary creatinine (U_{Alb}/U_{Cr}) (Equation 5) were measured at 8 and 18 months in seven children in the control group and six children in the low protein group (Table 2). Microproteinuria did occasionally occur in both groups. The results, however, were too few to allow meaningful comparisons between the diet groups.

Discussion

The method we developed for measuring the plasma C_{Io}, using an extended constant intravenous infusion of iothalamate

Table 2. Albumin excretion of patients in the control and low groups

Pt #	Θ Alb 8 months	Θ Alb 18 months	Diff 18–8	Alb/Cr[a] 8 months	Alb/Cr 18 months	Diff 18–8
	$\times 10^{-5}$			*mg/mg*		
C-1	6	63	57	0.08	2.10	2.02
C-2	24	33	9	0.73	0.77	0.04
C-8	5	2	−3	0.18	0.05	−0.13
C-12	9	9	0	0.29	0.26	−0.03
C-14	15	14	−1	0.36	0.23	−0.13
C-19	7	6	−1	0.23	0.13	−0.10
C-23	39	8	−31	2.30	0.38	−1.92
L-3	10	17	7	0.23	0.08	−0.15
L-5	47	5	−42	0.53	0.09	−0.44
L-7	96	14	−82	0.22	0.03	−0.19
L-13	9	8	−1	0.22	0.29	0.07
L-16	120	16	−104	3.60	0.30	−3.30
L-17	16	5	−11	0.34	0.11	−0.23

[a] Mean and range (Barratt: *Ped Neph* p 283) for normal 0.08 mg/mg (0.02–0.03 mg/mg).

to steady state, is a satisfactory alternative to the plasma C_{In} as described by Cole et al [14] and to single injection methods. We describe calculations we used to estimate clearance, infusion rate, minimum infusion time and whether a steady state had been reached. In estimating clearance from the ht/S_{Cr} formula (est GFR, Equation 1) for serial studies in this age range we decided to use the constant 0.45 for each age rather than 0.55 for children past one year of age as is recommended [15]. Tubular secretion of creatinine tends to increase as C_{Cr} declines [12]; using the smaller constant empirically adjusts for this tendency. We decided to use iothalamate because it is easier to use, less expensive and more consistently available than inulin. In our experience and the experience of other investigators [22–24] no clinically significant difference has been noted between either the renal or plasma C_{Io} and C_{In}. In one report [25] clearances in a few humans were lowered when probenecid was given, suggesting that a small fraction of iothalamate was excreted by tubular secretion. A similar effect of probenecid upon inulin clearance also was reported, casting some question on the inference that tubular secretion had occurred. The single injection method using four or fewer samples has a relatively high coefficient of variation and is systematically higher because of the gradient between plasma and interstitial fluid that persists in single injection clearance studies [26]. Further, the method is not well suited to infants because it is difficult to get frequent precisely timed blood samples.

Our protocol when used in the different centers of the multicenter trial was adhered to with little variation. With training and experience, research nurses were able to complete 71 of 74 clearance studies satisfactorily; of the 71 there were two in which the S_1 to S_{Io} ratio was outside allowances and a steady state may not have been reached. Θ Albumin was readily determined by collecting an *untimed* urine sample during the period when S_{Io} was near steady state, and measuring iothalamate and albumin on the serum and untimed urine sample. The method is suitable for estimating GFR where difficulties preclude the measuring of renal clearances. It has advantages over the single injection method in that its accuracy is greater and can be verified and fractional clearances of albumin, or other substances can be simply done.

The protocol resulted in substantial differences in dietary protein intake between the study groups. These differences were sufficient to test whether a low versus an average, but not high, protein intake slowed progression of renal insufficiency. There were no differences in calorie intake. The lack of significant difference in change of GFR between the study groups should not be regarded as evidence that dietary protein differences in this range have no effect. The small number of patients in the study and the short period of observation make it impractical to draw conclusions on that point from this preliminary study. A study using more infants and following each for a longer period of time is needed to test the hypothesis that lowering dietary protein to the *minimum requirement for normal children* as opposed to the average alters the rate of change in GFR in infants with reduced renal function. Our findings indicate that such a study is feasible. Until the question is resolved, we caution against using very low protein diets.

The findings reaffirm the insensitivity of S_{Cr} and C_{Cr}, whether derived from a ht/S_{Cr} equation or directly measured, as estimates of GFR and encourage the use of more suitable methods. The finding of microalbuminuria and the relative ease with which Θ_{Alb} can be derived when plasma clearance is measured by constant infusion suggest this may be another useful indicator for assessing glomerular injury.

The significant findings were the excellent agreement between the C_{Io} and the C_{In} and, in the feasibility study, the increase in absolute GFR (ml/min) that occurred between 8 and 18 months of age. The relative increase was observed as frequently in those whose reductions in GFR was severe (< 25% nl) as with those whose reductions in GFR were moderate (25 to 50% nl). We conclude that the increase in this period was more a function of developmental stage of the infant than an indication of the potential for recovery; an increase in absolute GFR in this age group should be cautiously interpreted as a sign of recovery. Adjusting GFR to % nl for age and weight rather than to 1.73 m^2 gives a more accurate picture of the course of GFR in this period of growth when GFR is increasing at a faster rate than is SA.

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